Evaluation of APOE Genotype and Ability to Perform Activities of Daily Living Following Aneurysmal Subarachnoid Hemorrhage

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Abstract

Survivors of aneurysmal subarachnoid hemorrhage (aSAH) often experience unfavorable functional outcomes that result in a reduced ability to independently perform activities of daily living. The apolipoprotein E gene (*APOE*) encodes for a protein known to facilitate lipid transport and aid in neuronal repair within the central nervous system and to moderate the inflammatory response, making functional variations in this gene likely candidate biomarkers to predict outcomes following aSAH. In the present work, we examined the relationship between *APOE* genotype and the ability to perform activities of daily living as measured by the Barthel Index (BI) score at 3 months ($n = 298$) and 12 months ($n = 288$) following aSAH. *APOE* genotypes were determined using polymerase chain reaction followed by restriction digestion and gel electrophoresis and treated as binary variables depending on the presence or absence of alleles E4 and E2. Multiple logistic regression was used to determine whether *APOE* genotype accounted for variability in BI score after controlling for age, sex, and severity of clinical condition as measured by the Hunt and Hess classification. No significant association was found between the presence of allele E4 and BI score at 3 ($p = .20$) or 12 months ($p = .29$) or between the presence of allele E2 and BI score at 3 ($p = .23$) or 12 months ($p = .86$) after controlling for covariates. The results of this study do not support a relationship between *APOE* genotype and the ability to perform activities of daily living after aSAH.

Keywords

subarachnoid hemorrhage, apolipoprotein E, genetics, polymorphism, Barthel Index score, activities of daily living

Aneurysmal subarachnoid hemorrhage (aSAH) is a devastating form of stroke with a disconcertingly young mean age of onset of around 55 years. Mortality rates for aSAH approach 45–50% within the first 30 days, and those who do survive are often left with severe functional, cognitive, and emotional deficits that make daily functioning difficult (van Gijn, Kerr, & Rinkel, 2007). The inability to perform activities of daily living leaves these survivors greatly dependent on caregivers and with a reduced quality of life (Al-Khindi, MacDonald, & Schweizer, 2010). While predictors of unfavorable outcomes after aSAH exist, including initial extent of bleed and the development of delayed cerebral ischemia (Teo et al., 2017), clinicians remain unable to comprehensively identify all patients at risk. This gap in knowledge impedes the development of personalized therapeutic interventions, rehabilitation provisions, and long-term care planning to maximize outcomes for this population.

Recent data suggest that inflammatory mechanisms and genetic variations may contribute to poor outcomes following aSAH (Chaudhry et al., 2017). Specifically, research has shown

that variants in the apolipoprotein E gene (*APOE*) play an important role in moderating patient functional and neuropsychological outcomes following several forms of neurologic injury, including intracerebral hemorrhage (James, Blessing, Bennett, & Laskowitz, 2009), traumatic brain injury (Li et al., 2015), and subarachnoid hemorrhage (Lanterni et al.,

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2007). Perhaps most recognized for its strong influences in mild cognitive impairment, Alzheimer's disease, and dementia (Viticchi et al., 2014), variation in *APOE* is widely accepted to play a strong role in cerebrovascular diseases. Although the exact mechanism of *APOE* involvement in the pathological processes following aSAH is unclear, the protein for which *APOE* encodes is well-known as a potent facilitator of lipid transport and neuronal repair, opponent of oxidative stress, and isoform-specific regulator of inflammatory responses (Colton, Brown, Czapiga, & Vitek, 2002) within the central nervous system.

Three major *APOE* alleles (E2, E3, and E4) are inherited as six common *APOE* genotypes (*APOE* 2/2, *APOE* 2/3, *APOE* 2/4, *APOE* 3/3, *APOE* 3/4, and *APOE* 4/4; Mahley, 1988). The antioxidant ability of *APOE* is isoform-specific, with E2 having the highest antioxidant potential. In contrast, E4 has the lowest antioxidant potential, suggesting the potential for patients with this allele to be less likely to release lipids for neuronal repair compared to those with other isoforms (Jolivald et al., 2000). Although numerous studies have examined the role of *APOE* genotype in patient outcomes following aSAH, with mixed results, the relationship between *APOE* genotype and the ability to perform activities of daily living following aSAH specifically has received less attention. This gap in knowledge is important, as potential modification of care plans based on genotype has widespread nursing implications as the science moves toward personalized medicine.

Material and Method

Sample

We prospectively recruited participants for this exploratory genetic study as part of an ongoing National Institutes of Health-funded study ("the parent project") approved by the University of Pittsburgh Institutional Review Board. Participants were recruited from UPMC Presbyterian Hospital in Pittsburgh, PA, and were included in the parent project if they were between the ages of 18 and 75 years, had been diagnosed with aSAH verified with cerebral angiogram, and were able to read/speak English. Participants were excluded if they had a history of a debilitating neurological disorder or if their subarachnoid hemorrhage resulted from trauma, arteriovenous malformation, or mycotic aneurysm. Participants undergoing clinical data collection for the parent project were concurrently consented for the collection of a repository genetic sample. We obtained consent from a legal proxy if the participant was unable to provide personal consent. Participant demographics and clinical data, including age, sex, and severity of aSAH (as measured by the clinical grading system, the Hunt and Hess [HH] classification), were extracted from the medical record.

Genotype data were available for 524 participants. We excluded 116 participants from the present analysis due to missing outcome data at both 3 and 12 months. Given that allele frequencies differed by ancestry, we also excluded 62 non-Caucasian participants to control for population stratification, significantly reducing the chance of spurious findings

based solely on the underlying structure of the population as opposed to an actual association with our locus of interest (Little et al., 2009). The non-Caucasian group was not large enough for the conduct of subgroup analyses. The final sample consisted of 346 Caucasian participants; sample size varied at cross-sectional outcome time points based on availability of outcome data (at 3 months, $n = 298$; at 12 months, $n = 288$).

Genotyping

We performed *APOE* genotyping using DNA extracted from either cerebrospinal fluid or whole blood collected at recruitment, as the DNA template will not vary depending on the tissue used. DNA was extracted using a simple salting-out procedure (Miller, Dykes, & Polesky, 1988), and genotypes were determined using polymerase chain reaction followed by restriction digestion and gel electrophoresis. These genotypes were compared to genetic ladders and to standards of known, sequenced genotypes. Individuals blinded to phenotypes double called all genotypes, and we resolved genotype discrepancies by retyping any disputed samples.

Outcome Phenotyping

A trained neuropsychiatric technician or a nurse trained in neuropsychiatric testing evaluated participants' ability to perform activities of daily living via face-to-face or telephone interview at 3 and 12 months following aSAH using the Barthel Index (BI). The BI score is a composite measure of the ability to perform activities of daily living including functions such as toileting, grooming, dressing, mobility, and transfer. The interviewer rated items on a scale ranging from 0 (*death or unable to perform*) to 2 (*can independently perform*) to produce a composite score ranging from 0 to 20, with lower scores indicating lesser ability to perform activities of daily living. The BI score has demonstrated excellent test-retest reliability (Cohen's $\kappa = .41$ – 1.00) and internal consistency (Cronbach $\alpha = .80$ – $.93$) in stroke patients (Quinn, Langhorne, & Stott, 2011). Importantly, telephone administration has a reliability comparable to that of face-to-face administration (Della Pietra et al., 2011). Given that some participants were impaired, interviewers administered the BI questionnaire to either the participant or the primary caregiver.

Statistical Analysis

We carried out all statistical analyses using SPSS, Version 24 (IBM, Chicago, IL). First, we conducted a preliminary analysis to evaluate relationships between variables, compute detailed descriptive statistics, examine data graphically, and investigate sources of missing or extreme data. Given the distributions of genotypes within this sample, genotype subgroup analyses were not possible. We treated genotypes as binary variables and classified them first based on the presence or absence of at least one *APOE* E4 allele (*APOE* 2/4, *APOE* 3/4, and *APOE* 4/4 vs. *APOE* 2/2, *APOE* 2/3, and *APOE* 3/3) and again based

Table 1. Demographics and Univariate Analysis to Evaluate Relationships Between Variables Based on Presence or Absence of *APOE* Alleles E4 and E2.

Variable	Sample N = 346	APOE E4		p Value	APOE E2		p Value
		E4 Present (APOE 2/4, APOE 3/4, APOE 4/4)	E4 Absent (APOE 2/2, APOE 2/3, APOE 3/3)		E2 Present (APOE 2/2, APOE 2/3, APOE 2/4)	E2 Absent (APOE 3/3, APOE 3/4, APOE 4/4)	
		n = 84	n = 262		n = 42	n = 304	
Age (years)				.45			.67
Mean (SD)	53.9 (11.3)	54.8 (12.2)	53.7 (11.0)		54.6 (10.9)	53.8 (11.4)	
Range	18–75	18–75	25–75		33–75	18–75	
Sex, female, n (%)	239 (69.1)	62 (25.9)	177 (74.1)	.28	30 (12.6)	209 (87.4)	.73
HH grade, n (%)				.62			.41
1–2	169 (48.8)	43 (25.4)	126 (74.6)		18 (10.7)	151 (89.3)	
3–5	177 (51.2)	41 (23.2)	136 (76.8)		24 (13.6)	153 (86.4)	

Note. HH = Hunt and Hess grade; *APOE* = apolipoprotein E gene; SD = standard deviation.

Table 2. Univariate Analysis to Evaluate Relationships Between Demographic Variables and HH Grades and Outcome, as Measured by the 3- and 12-Month BI Scores.

Variable	Sample N = 346	3-Month BI Score		p Value	12-Month BI Score		p Value
		Unfavorable n = 75	Favorable n = 223		Unfavorable n = 59	Favorable n = 229	
Age (years)				.003			.10
Mean (SD)	53.9 (11.3)	56.9 (11.4)	52.8 (10.9)		56.4 (11.9)	54.3 (11.2)	
Range	18–75	18–75	24–75		18–75	24–75	
Sex, female, n (%)	239 (69.1)	54 (26.5)	150 (73.5)	.45	41 (20)	164 (80)	.75
HH grade, n (%)				<.001			<.001
1–2	169 (48.8)	14 (9.9)	128 (90.1)		9 (6.3)	134 (93.7)	
3–5	177 (51.2)	61 (39.1)	95 (60.9)		50 (34.5)	95 (65.5)	

Note. Unfavorable BI scores were in the range 0–18; favorable BI scores were in the range 19–20. HH = Hunt and Hess grade; BI = Barthel Index; SD = standard deviation.

on the presence or absence of at least one *APOE* E2 allele (*APOE* 2/2, *APOE* 2/3, *APOE* 2/4 vs. *APOE* 3/3, *APOE* 3/4, and *APOE* 4/4). Similarly, use of the full range of BI score values was not possible given the sample size and skewness of the data. Therefore, we dichotomized the BI score as unfavorable (0–18) versus favorable (19–20). We chose a range of ≥ 19 to indicate a favorable outcome because it is the optimum cut-off score for excellent recovery after stroke, corresponding to a modified Rankin Scale score of 1 (sensitivity 85.6%; specificity 91.7%; Uyttenboogaart, Stewart, Vroomen, De Keyser, & Luijkx, 2005). We dichotomized the covariate HH grade as low (1 or 2) or high (3, 4, or 5) based on previously reported differences in patient outcomes in these groups (Lantigua et al., 2015). We evaluated associations between genotype groups and BI score using logistic regression. In the final stage of analysis, our statistical modeling considered genotype groups separately to yield unadjusted regression coefficients, and we then expanded it to yield adjusted estimates controlling for age, sex, and HH grade. We considered results to be statistically significant if the *p* value was $\leq .05$.

Results

Participants in the present study had a mean age of 53.9 years and were 69.1% female. Age, sex, and HH grade for participants in this study did not differ significantly from those of the parent project. Participant demographics and HH grades are presented for the total sample and by allele group in Table 1. *APOE* genotype distribution showed *APOE* 3/3 as the most common (65.6%), followed by *APOE* 3/4 (21.4%), *APOE* 2/3 (10.1%), *APOE* 2/4 (2%), and *APOE* 4/4 (0.9%). The *APOE* 2/2 genotype did not occur in the present sample, but baseline demographic and clinical data did not differ between *APOE* groups.

Next, we conducted a preliminary analysis to evaluate the relationships between outcome, as measured by BI scores, and both demographics and HH grades. We present the results in Table 2. The percentage of unfavorable BI scores at 3 and 12 months was 25.2% and 20.5%, respectively. Increased age was associated with unfavorable BI score at 3 months (*p* = .003) but not at 12 months (*p* = .10). Higher HH grade was

Table 3. Results From Univariate and Multivariate Analyses Exploring Associations Between Presence of *APOE* Alleles E4 and E2 With 3- and 12-Month BI Score.

Variable	<i>n</i>	APOE E4 Present (24.3%; APOE 2/4, APOE 3/4, APOE 4/4 vs. APOE 2/2, APOE 2/3, APOE 3/3)			APOE E2 Present (12.1%; APOE 2/2, APOE 2/3, APOE 2/4 vs. APOE 3/3, APOE 3/4, APOE 4/4)		
		OR	95% CI	<i>p</i> Value	OR	95% CI	<i>p</i> Value
3-Month BI	298	.73	[0.38, 1.38]	.33	.71	[0.31, 1.62]	.42
12-Month BI	288	.66	[0.32, 1.36]	.26	.97	[0.40, 2.34]	.94
Adjusted estimate ^a							
3-Month BI	298	.64	[0.32, 1.27]	.20	.58	[0.24, 1.40]	.23
12-Month BI	288	.66	[0.31, 1.43]	.29	.92	[0.34, 2.36]	.86

Note. BI = Barthel Index; *APOE* = apolipoprotein E gene; CI = confidence interval; OR = odds ratio.

^aAdjusted estimate controlling for age, sex, and Hunt and Hess grade.

associated with unfavorable BI score at 3 months ($p < .001$) and 12 months ($p < .001$). In contrast, sex was not associated with the ability to perform activities of daily living at 3 months ($p = .45$) or 12 months ($p = .75$).

Finally, we evaluated associations between *APOE* genotype and ability to perform activities of daily living and present the results in Table 3. We found no significant association between the presence of allele E4 and BI score at 3 months ($p = .20$) or 12 months ($p = .29$) after controlling for age, sex, and HH grade. Likewise, we found no significant association between the presence of allele E2 and BI score at 3 months ($p = .23$) or 12 months ($p = .86$) after controlling for age, sex, and HH grade.

Discussion

While *APOE* genotype has surfaced as an important predictor of select patient outcomes following traumatic brain injury (Li et al., 2015), intracerebral hemorrhage (James et al., 2009), and aSAH (Lantern et al., 2007), the findings of the present ancillary genetic study do not support a relationship between *APOE* genotype and the ability to perform activities of daily living as measured by BI score following aSAH. Our work is in agreement with data from a study involving a combined population of ischemic and hemorrhagic stroke victims in which investigators found no relationship between *APOE* genotype and BI score (Wagle et al., 2010). Importantly, a similar analysis in a smaller sample size of aSAH patients corroborated the results of this study (Csajbok et al., 2016).

The lack of a definitive association between *APOE* genotype and patient outcomes suggests that any relationship that may exist is part of a complex network moderated by many factors. It is possible that the link between *APOE* genotype is more strongly linked to functional impairment via cognitive changes (Andrews, Das, Cherbuin, Anstey, & Easta, 2016) and that a direct relationship between genotype and physical changes relevant to the ability to perform activities of daily living may not exist. Furthermore, the outcome measure that we selected for examination in this study provided a mean measure of ability to perform activities of daily living but did not examine additional measures of functional outcomes such as the Glasgow Outcome Scale (GOS), which researchers have

found to be associated with *APOE* genotype following aSAH (Lantern et al., 2007) and intracerebral hemorrhage (James et al., 2009). Although both instruments are well established measures of functional outcome, the BI Scale asks a multitude of questions specific to everyday activities, while the GOS is a gross measure of functional outcome with a smaller range of possible scores (1–5). While the scopes of both tools are important considerations for quality of life for aSAH patients, the results of the present study suggest that these outcomes could have very different biological underpinnings. Thus, it is possible that our results would be different if we had considered GOS score rather than BI score for our primary outcome measure. It is also possible that a self-report of one's ability to perform activities of daily living, like the BI, could be biased, with patients tending to overestimate their abilities (Shih, Rogers, Skidmore, Irrgang, & Holm, 2009). Lastly, in a review of 15 stroke trials, authors reported a remarkable difference in the choice of cutoff points for defining favorable BI scores compared with other measures of functional outcomes following stroke (Sulter, Steen, & De Keyser, 1999), which significantly hinders the comparison of the results of the present study with those of other studies examining the influence of *APOE* genotype on additional patient outcomes.

While the present study contributes to the existing literature finding no association between *APOE* genotype and the ability to perform activities of daily living following aSAH, there are several important limitations that we should acknowledge. First, the contribution of *APOE* genotype to recovery and the ability to perform activities of daily living in aSAH patients may have a small effect size that is not detectable in this sample. We selected a BI cutoff score of ≥ 19 to distinguish those with excellent recovery from those with dysfunction in ability to perform activities of daily living. Use of the full range of values, while not possible given this sample size, might show finer differences based on genotype. Our sample had many subjects without dysfunction in the ability to perform activities of daily living, which may have impaired our ability to identify these finer differences. Furthermore, our sample includes an insufficient number of homozygous risk alleles for subgroup analyses by all possible *APOE* genotypes separately. Specifically, there is underrepresentation of the two genotypes

reported to be most influential, *APOE* 2/2 and *APOE* 4/4. Given that isoform-specific proteins have different antioxidant ability (Jolivald et al., 2000), it is likely that each isoform is uniquely relevant in the pathological changes that occur within the central nervous system following aSAH. It may be that one copy of the common *APOE* 3 allele is adequate to support neuronal recovery, and we were therefore unable to detect dose-specific effects. It is also possible that people with homozygous E4 alleles could experience a higher early mortality rate and are, therefore, less likely to be admitted to the neurovascular intensive care unit to begin with, thus biasing this study. The present study was further limited in that it included a self-reported Caucasian sample. Future efforts are needed to examine this relationship in patients with more diverse ancestries, an especially crucial requirement considering the nonrandom geographical distribution of the E4 allele as well as cultural factors important to aSAH, including diet (Huebbe & Rimbach, 2017). Lastly, a majority of the sample examined in this study was female (69.1%), which is consistent with the reported demographics of the aSAH patient population (Kongable et al., 1996). While we found no association between sex and BI score and did control for this in the final analysis, there may be associations that are important only when stratified by sex.

It is a strength of this study that we have a relatively large sample size as compared to other work in the area (Csajbok et al., 2016). Our findings also indicated that age and HH grade together are strong predictors of future ability to perform activities of daily living, which are consistent with the literature. Information such as this is critically needed given the heterogeneity of outcomes following aSAH. Despite the null genotypic findings of this study, there remains a need for further exploration of genotype and gene–gene interactions as predictors of outcome variability for these patients. Genetic biomarkers hold great promise for impacting nursing science in the future. By identifying aSAH patients at risk for unfavorable outcomes, genetic biomarkers can aid nurses in (1) targeting these patients with early personalized therapeutic interventions to mitigate unfavorable outcomes, (2) developing detailed discharge education materials to caution patients and families about potential or likely complications, and (3) care planning for the future to implement comprehensive rehabilitation plans as early as possible.

Authors' Note

The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Author Contribution

L. Heinsberg contributed to conception, design, analysis, and interpretation; drafted the manuscript; critically revised the manuscript; gave final approval; and agrees to be accountable for all aspects of work, ensuring integrity and accuracy. E. Turi contributed to conception, design, analysis, and interpretation; critically revised the manuscript; gave final approval; and agrees to be accountable for all aspects of work, ensuring integrity and accuracy. D. Ren contributed to

analysis and interpretation; critically revised the manuscript; gave final approval; and agrees to be accountable for all aspects of work, ensuring integrity and accuracy. E. Crago contributed to acquisition and interpretation; critically revised the manuscript; gave final approval; and agrees to be accountable for all aspects of work, ensuring integrity and accuracy. S. Alexander contributed to acquisition and interpretation; critically revised the manuscript; gave final approval; and agrees to be accountable for all aspects of work, ensuring integrity and accuracy. A. Stanfill contributed to conception, design, and interpretation; critically revised the manuscript; gave final approval; and agrees to be accountable for all aspects of work, ensuring integrity and accuracy. Y. Conley contributed to conception, design, acquisition, and interpretation; critically revised the manuscript; gave final approval; and agrees to be accountable for all aspects of work, ensuring integrity and accuracy.

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